TENT COOPERATION TRE

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
17810-511		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/05840	03/03/2000	05/03/1999
International Patent Classification (IPC) or national classification and IPC C12N5/02		
Applicant CALIFORNIA INSTITUTE OF TECHNOLOGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the International application

Date of submission of the demand	Date of completion of this report
05/10/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Schwachtgen, J-L Telephone No. +49 89 2399 8933

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/05840

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-33 as originally filed

Claims, No.:

1-35 with telefax of 16/05/2001

Drawings, sheets:

1/2,2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-35
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-35
Industrial applicability (IA)	Yes:	Claims	1-11, 13-15, 17-35
	No:	Claims	

2. Citations and explanations
see separate sheet

R It m V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: WO 98 48001 A (CALIFORNIA INST OF TECHN) 29 October 1998 (1998-10-29)

2. Document D1 discloses a method for enriching a population of neural crest stem cells comprising contacting an "uncultured" single cell suspension made from a tissue containing neural crest stem cells with antibodies specific for LNGFR, which is identical to p75 of the pesent application, and isolating the LNGFR+ cells (page 28, line 12 to page 29, line 26). Neural crest stem cells can be further selected by the absence of the myelin protein P0 (page 16, lines 10-19). The step of culturing the isolated neural crest cells before enrichment is clearly identified as an optional embodiement only (page 30, lines 8-11) and it would also be recognised as such by the skilled person.

   The applicant's argument, that the subject-matter of amended claim 1 is distinguished from the disclosure in D1 by the feature that the selected cells are enriched to at least 50% of neural stem cells, cannot be accepted.
   In the decision T 990/96 (OJ EPO 1998, p. 489) of the boards of appeal of the EPO, it had to be examined whether the feature under dispute, which represented a specific degree of chemical purity constituted a "new element" imparting novelty to the claimed subject-matter. The conclusion was that, in general, a document disclosing a compound and its manufacture made this compound available to the public within the meaning of Art. 54 EPC (Article 33(2) PCT) in all grades of purity as desired by a person skilled in the art. A different conclusion could only arise where all prior attempts to achieve a particular degree of purity by conventional purification processes had failed. In the present case, however, D1 discloses neural crest stem cells purified from neural tissue by means of antibodies specific for LNGFR and standard published cell isolation procedures used with other antibodies and cells to achieve more than 50% of enrichment.

D1, thus anticipates all the technical features of independ nt claims 1 and 22 of the present application, contrary to the requirements of Articl 33(2) PCT. The same objection applies to claims 2-21 and 23-35.

3. Claims 12 and 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

CLAIMS

1. A method for enriching a population of uncultured cells for neural stem cells, comprising:
   (a) obtaining a population of uncultured cells containing a neural stem cell by dissociating intact tissue,
   (b) contacting said population with a combination of reagents, wherein each reagent in the combination either selectively binds to either a neural stem cell positive marker or a neural stem cell negative marker; and
   (c) selecting the cells which bind to the reagents that selectively bind to a positive marker or the cells which do not bind to the reagents that selectively bind to a negative marker, or a combination thereof, wherein the selected cells are enriched to at least 50% in neural stem cells as compared with the population of uncultured cells.

2. The method of claim 1, wherein the neural stem cell is a neural crest stem cell (NCSC).

3. The method of claim 1, wherein the neural stem cell is a central nervous system (CNS) neural stem cell.

4. The method of claim 1, wherein the selected cells are at least 50% neural stem cells.

5. The method of claim 1, wherein the reagents in step (b) are antibodies.

6. The method of claim 1, wherein the reagent that selectively binds to a positive marker is an anti-p75 (low-affinity neurotrophin receptor) antibody.

7. The method of claim 1, wherein the reagent that selectively binds to a negative marker is an anti-P_0 antibody.

8. The method of claim 1, wherein the population of uncultured cells is obtained from the neural crest.

9. The method of claim 1, wherein th population of uncultured cells is dissociated neural tissue.

10. The method of claim 1, wherein the population of uncultured cells is dissociated peripheral nerve.

11. The method of claim 1, wherein the selecting is by flow cytometry.

12. The method of claim 1, further comprising:

(d) transplanting the selected cells into a host.

13. A method for enriching a population of uncultured cells for neural stem cells, comprising:

(a) contacting a population of cells containing a fraction of neural stem cells with a reagent that specifically binds to p75 (low-affinity neurotrophin receptor); and

(b) selecting $p75^+$ cells, wherein the selected $p75^+$ cells are enriched to at least 50% neural stem cells as compared with the unselected population of cells.

14. The method of claim 13, further comprising:

(c) contacting the selected $p75^+$ cells with a reagent that specifically binds to the P_0 antigen; and

(d) selecting P_0^- cells, wherein the selected $p75^+$ P_0^- cells are enriched in the fraction of neural stem cells as compared with the population of neural cells.

15. A method for isolating a neural stem cell, comprising:

(a) contacting a population of uncultured cells containing a neural stem cell with a combination of reagents, wherein each reagent in the combination either selectively binds to either a neural stem cell positive marker or a neural stem cell negative marker;

(b) selecting cells which bind to reagents that selectively bind to a positive marker or which do not bind to reagents that selectively bind to a negative marker or a combination thereof;

(c) introducing at least one selected cell to a culture medium, which supports the growth of neural stem cells; and

(d) proliferating the selected cell in the culture medium, wherein the proliferated progeny cells are obtained from an isolated neural stem cell.

16. The method of claim 15, wherein the culture medium which supports the growth of neural stem cells comprises a serum free-medium containing chick embryo extract.

17. The method of claim 15, further comprising:

(e) differentiating the proliferated progeny cells to produce a cell culture comprising differentiated cells selected from the group consisting of neurons, glia, myofibroblasts, and combinations thereof.

18. The method of claim 15, further comprising:

(e) transplanting the proliferated progeny cells into a host.

19. The method of claim 15, further comprising:

(e) contacting the proliferated progeny cells with a biological agent; and

(f) determining the effects of the biological agent on the proliferated progeny cells.

20. The method of claim 15, further comprising:

(e) inducing the proliferated progeny cells to differentiate in a second culture medium containing a biological agent; and

(f) determining the effects of the biological agent on the differentiated cells.

21. The method of claim 15, further comprising:

(e) inducing the proliferated progeny cells to differentiate in a second culture containing a biological agent;

(f) contacting the differentiated cells with the biological agent; and

(g) determining the effects of the biological agent on the differentiated neural cells.

22. An *in vitro* cell culture composition, comprising:

(a) a population of uncultured cells enriched for neural stem cells according to the method of claim 1; and

(b) a culture medium that supports the growth of neural stem cells.

23. The composition of claim 22, wherein the population of uncultured cells are derived from dissociated nerves.

24. The composition of claim 22, wherein the population of uncultured cells are derived from primary peripheral nervous system tissue.

25. The composition of claim 22, wherein the population of uncultured cells are derived from primary central nervous system tissue.

26. The composition of claim 22, wherein the population of uncultured cells are derived by immunoselection using an anti-p75 (low-affinity neurotrophin receptor) antibody.

27. The composition of claim 22, wherein the population of uncultured cells are derived by immunoselection using an anti-P_0 antibody.

28. The composition of claim 22, wherein the population of uncultured cells has at least 80% $p75^+$ cells.

29. The composition of claim 22, wherein the neural stem cells are from a rat.

30. The composition of claim 22, wherein the neural stem cells are from a chick.

30. The composition of claim 22, wherein the neural stem cells are from a human.

32. The composition of claim 22, wherein the culture medium comprises a serum free-medium containing chick embryo extract.

33. The composition of claim 22, wherein the culture medium comprises an instructive factor.

34. The composition of claim 33, wherein the instructive factor is a growth factor of the TGF-β superfamily.

35. The composition of claim 33, wherein the instructive factor is a neuregulin (NRG-1).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
17810-511		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/05840	03/03/2000	05/03/1999
Applicant		
CALIFORNIA INSTITUTE OF TECHNOLOGY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawing** to be published with the abstract is Figure No. _____

   [X] None of the figures.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

International Application No
PCT/US 00/05840

A. CLASSIFICATION OF SUBJECT MA
IPC 7 C12N5/02 C12N5/06 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 48001 A (CALIFORNIA INST OF TECHN) 29 October 1998 (1998-10-29)	1-13, 15-19, 22-27, 29-35
A	abstract page 11, line 1 - line 24 page 15, line 12 - line 18 page 16, line 1 - line 19 page 24, line 11 - line 20 page 26, line 4 - line 22 page 27, line 9 - line 11 page 28, line 12 -page 29, line 20 page 32, line 6 - line 10 page 32, line 24 -page 33, line 4 page 34, line 26 -page 35, line 20 page 41, line 6 - line 9 page 44, line 15 - line 23 example 11 --- -/--	14,20,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2000	20/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Noë, V

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	STEMPLE D L ET AL: "ISOLATION OF A STEM CELL FOR NEURONS AND GLIA FROM THE MAMMALIAN NEURAL CREST" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 71, no. 11, 11 December 1992 (1992-12-11), pages 973-985, XP002063900 ISSN: 0092-8674 cited in the application abstract the whole document	
A	SHAH NIRAO M ET AL: "Alternative neural crest cell fates are instructively promoted by TGF-beta superfamily members." CELL, vol. 85, no. 3, 1996, pages 331-343, XP002074389 ISSN: 0092-8674 cited in the application the whole document	33-35
P,X	MORRISON SEAN J ET AL: "Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells." CELL, vol. 96, no. 5, 5 March 1999 (1999-03-05), pages 737-749, XP002149096 ISSN: 0092-8674 the whole document	1-35
P,X	UCHIDA N ET AL: "Direct isolation of human neural stem cells from fetal brain by cell sorting." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 25, no. 1-2, 1999, page 1767 XP002149097 29th Annual Meeting of the Society for Neuroscience.;Miami Beach, Florida, USA; October 23-28, 1999 ISSN: 0190-5295 abstract	1,11,12, 15,17, 18,30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/05840

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12 and 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Information on patent family members

International Application No
PCT/US 00/05840

Patent document cited in search report	Publication dat	Patent family member(s)	Publication dat
WO 9848001 A	29-10-1998	US 6001654 A	14-12-1999
		AU 7258098 A	13-11-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
17810-511		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/05840	03/03/2000	05/03/1999
International Patent Classification (IPC) or national classification and IPC C12N5/02		
Applicant CALIFORNIA INSTITUTE OF TECHNOLOGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

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3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/10/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Schwachtgen, J-L Telephone No. +49 89 2399 8933

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/05840

I. Basis f th r p rt

1. With regard to the **lem nts** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-33 as originally filed

Claims, No.:

1-35 with telefax of 16/05/2001

Drawings, sheets:

1/2,2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
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3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-35
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-35
Industrial applicability (IA)	Yes:	Claims	1-11, 13-15, 17-35
	No:	Claims	

2. Citations and explanations
   see separate sheet

R Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: WO 98 48001 A (CALIFORNIA INST OF TECHN) 29 October 1998 (1998-10-29)

2. Document D1 discloses a method for enriching a population of neural crest stem cells comprising contacting an "uncultured" single cell suspension made from a tissue containing neural crest stem cells with antibodies specific for LNGFR, which is identical to p75 of the pesent application, and isolating the LNGFR+ cells (page 28, line 12 to page 29, line 26). Neural crest stem cells can be further selected by the absence of the myelin protein P0 (page 16, lines 10-19). The step of culturing the isolated neural crest cells before enrichment is clearly identified as an optional embodiement only (page 30, lines 8-11) and it would also be recognised as such by the skilled person.

   The applicant's argument, that the subject-matter of amended claim 1 is distinguished from the disclosure in D1 by the feature that the selected cells are enriched to at least 50% of neural stem cells, cannot be accepted.
   In the decision T 990/96 (OJ EPO 1998, p. 489) of the boards of appeal of the EPO, it had to be examined whether the feature under dispute, which represented a specific degree of chemical purity constituted a "new element" imparting novelty to the claimed subject-matter. The conclusion was that, in general, a document disclosing a compound and its manufacture made this compound available to the public within the meaning of Art. 54 EPC (Article 33(2) PCT) in all grades of purity as desired by a person skilled in the art. A different conclusion could only arise where all prior attempts to achieve a particular degree of purity by conventional purification processes had failed. In the present case, however, D1 discloses neural crest stem cells purified from neural tissue by means of antibodies specific for LNGFR and standard published cell isolation procedures used with other antibodies and cells to achieve more than 50% of enrichment.

D1, thus anticipates all the technical features of independent claims 1 and 22 of the present application, contrary to the requirements of Article 33(2) PCT. The same objection applies to claims 2-21 and 23-35.

3. Claims 12 and 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

INTERNA NAL SEARCH REPORT

Int .tional Application No
PCT/US 00/05840

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/02 C12N5/06 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 48001 A (CALIFORNIA INST OF TECHN) 29 October 1998 (1998-10-29)	1-13, 15-19, 22-27, 29-35
A	abstract page 11, line 1 - line 24 page 15, line 12 - line 18 page 16, line 1 - line 19 page 24, line 11 - line 20 page 26, line 4 - line 22 page 27, line 9 - line 11 page 28, line 12 -page 29, line 20 page 32, line 6 - line 10 page 32, line 24 -page 33, line 4 page 34, line 26 -page 35, line 20 page 41, line 6 - line 9 page 44, line 15 - line 23 example 11 --- -/--	14,20,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2000	20/10/2000

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STEMPLE D L ET AL: "ISOLATION OF A STEM CELL FOR NEURONS AND GLIA FROM THE MAMMALIAN NEURAL CREST" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 71, no. 11, 11 December 1992 (1992-12-11), pages 973-985, XP002063900 ISSN: 0092-8674 cited in the application abstract the whole document	
A	SHAH NIRAO M ET AL: "Alternative neural crest cell fates are instructively promoted by TGF-beta superfamily members." CELL, vol. 85, no. 3, 1996, pages 331-343, XP002074389 ISSN: 0092-8674 cited in the application the whole document	33-35
P,X	MORRISON SEAN J ET AL: "Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells." CELL, vol. 96, no. 5, 5 March 1999 (1999-03-05), pages 737-749, XP002149096 ISSN: 0092-8674 the whole document	1-35
P,X	UCHIDA N ET AL: "Direct isolation of human neural stem cells from fetal brain by cell sorting." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 25, no. 1-2, 1999, page 1767 XP002149097 29th Annual Meeting of the Society for Neuroscience.;Miami Beach, Florida, USA; October 23-28, 1999 ISSN: 0190-5295 abstract	1,11,12, 15,17, 18,30

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[redacted]ional Application No
PCT/US 00/05840

Patent document cited in search report	Publication date	Patent family member(s)	Publication dat
WO 9848001 A	29-10-1998	US 6001654 A	14-12-1999
		AU 7258098 A	13-11-1998

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International filing date (day/month/year) 03 March 2000 (03.03.00)	**Priority date** (day/month/year) 05 March 1999 (05.03.99)
Applicant ANDERSON, David, J. et al	

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(54) Title: THE ISOLATION AND ENRICHMENT OF NEURAL STEM CELLS FROM UNCULTURED TISSUE BASED ON CELL-SURFACE MARKER EXPRESSION

(57) Abstract

The invention provides methods for the prospective identification, isolation, and self-renewal of neural stem cells from the mammalian peripheral nervous system and compositions of neural stem cells derived from uncultured tissue. Using flow-cytometry, neural crest derived cells of embryonic peripheral nerve were fractionated based on cell surface markers. The isolated $p75^+$ P_0 cells from embryonic sciatic nerve were phenotypically and functionally indistinguishable from neural crest stem cells (NCSCs) previously isolated from neural tube explant cultures. Furthermore, freshly isolated $p75^+$ P_0 cells gave rise to both neurons and glia when transplanted *in vivo*. Cell cycle analysis and BrdU labeling indicated that $p75^+P_0$ NCSCs persist in the peripheral nerve by undergoing self-renewing divisions after neural crest migration has ceased.

WE CLAIM:

1. A method for enriching a population of uncultured cells for neural stem cells, comprising:

   (a) contacting a population of uncultured cells containing a neural stem cell with a combination of reagents, wherein each reagent in the combination either selectively binds to a either neural stem cell positive marker or a neural stem cell negative marker; and

   (b) selecting cells which bind to reagents that selectively bind to a positive marker or which do not bind to reagents that selectively bind to a negative marker or a combination thereof, wherein the selected cells are enriched in neural stem cells as compared with the population of uncultured cells.

2. The method of claim 1, wherein the neural stem cell is a neural crest stem cell (NCSC).

3. The method of claim 1, wherein the neural stem cell is a central nervous system (CNS) neural stem cell.

4. The method of claim 1, wherein the selected cells are at least 50% neural stem cells.

5. The method of claim 1, wherein a reagent is an antibody.

6. The method of claim 1, wherein a reagent is an anti-p75 (low-affinity neurotrophin receptor) antibody.

7. The method of claim 1, wherein a reagent is an anti-P_0 antibody.

8. The method of claim 1, wherein the population of uncultured cells is derived from the neural crest.

9. The method of claim 1, wherein the population of uncultured cells is dissociated neural tissue.

10. The method of claim 1, wherein the population of uncultured cells is dissociated peripheral nerve.

11. The method of claim 1, wherein the selecting is by flow cytometry.

12. The method of claim 1, further comprising:

(c) transplanting the selected cells into a host.

13. A method for enriching a population of cells for the neural stem cell fraction, comprising:

(a) contacting a population of cells containing a fraction of neural stem cells with a reagent that specifically binds to p75 (low-affinity neurotrophin receptor); and

(b) selecting $p75^+$ cells, wherein the selected $p75^+$ cells are enriched in the fraction of neural stem cells as compared with the unselected population of cells.

14. The method of claim 13, further comprising:

(c) contacting the selected $p75^+$ cells with a reagent that specifically binds to the P_0 antigen; and

(d) selecting P_0^+ cells, wherein the selected $p75^+$ P_0^+ cells are enriched in the fraction of neural stem cells as compared with the population of neural cells.

15. A method for isolating a neural stem cell, comprising:

(a) contacting a population of uncultured cells containing a neural stem cell with a combination of reagents, wherein each reagent in the combination either selectively binds to either a neural stem cell positive marker or a neural stem cell negative marker;

(b) selecting cells which bind to reagents that selectively bind to a positive marker or which do not bind to reagents that selectively bind to a negative marker or a combination thereof;

(c) introducing at least one selected cell to a culture medium capable of supporting the growth of neural stem cells; and

(d) proliferating the selected cell in the culture medium, wherein the proliferated progeny cells are derived from an isolated neural stem cell.

16. The method of claim 15, wherein the culture medium capable of supporting the growth of neural stem cell comprises a serum free-medium containing chick embryo extract.

17. The method of claim 15, further comprising:

(e) differentiating the proliferated progeny cells to produce a cell culture comprising differentiated cells selected from the group consisting of neurons, glia, myofibroblasts, and combinations thereof.

18. The method of claim 15, further comprising:

(e) transplanting the proliferated progeny cells into a host.

19. The method of claim 15, further comprising:

(e) contacting the proliferated progeny cells with a biological agent; and

(f) determining the effects of the biological agent on the proliferated progeny cells.

20. The method of claim 15, further comprising:

(e) inducing the proliferated progeny cells to differentiate in a second culture medium containing a biological agent; and

(f) determining the effects of the biological agent on the differentiated cells.

21. The method of claim 15, further comprising:

(e) inducing the proliferated progeny cells to differentiate in a second culture containing a biological agent;

(f) contacting the differentiated cells with the biological agent; and

(g) determining the effects of the biological agent on the differentiated neural cells.

22. An *in vitro* cell culture composition, comprising:

(a) a population comprising at least 50% self-renewing multipotent neural stem cells, wherein the neural stem cells have been derived from uncultured tissue; and

(b) a culture medium that supports the growth of neural stem cells.

23. The composition of claim 22, wherein the population of cells are derived from dissociated nerves.

24. The composition of claim 22, wherein the population of cells are derived from primary peripheral nervous system (PNS) tissue.

25. The composition of claim 22, wherein the population of cells are derived from primary central nervous system (CNS) tissue.

26. The composition of claim 22, wherein the population of cells are derived by immunoselection using an anti-p75 antibody.

27. The composition of claim 22, wherein the population of cells are derived by immunoselection using an anti-P_0 antibody.

28. The composition of claim 22, wherein the population of cells has at least 80% $p75^-$ cells.

29. The composition of claim 22, wherein the neural stem cells are rat.

30. The composition of claim 22, wherein the neural stem cells are chick.

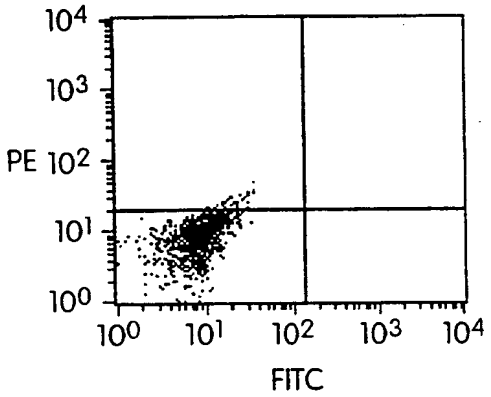
30. The composition of claim 22, wherein the neural stem cells are human.

32. The composition of claim 22, wherein the culture medium comprises a serum free-medium containing chick embryo extract.

33. The composition of claim 22, wherein the culture medium comprises an instructive factor.

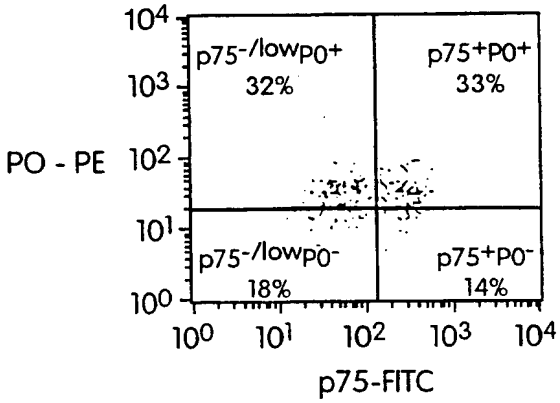
34. The composition of claim 33, wherein the instructive factor is a growth factor of the TGF-β superfamily.

35. The composition of claim 33, wherein the instructive factor is a neuregulin (NRG-1).



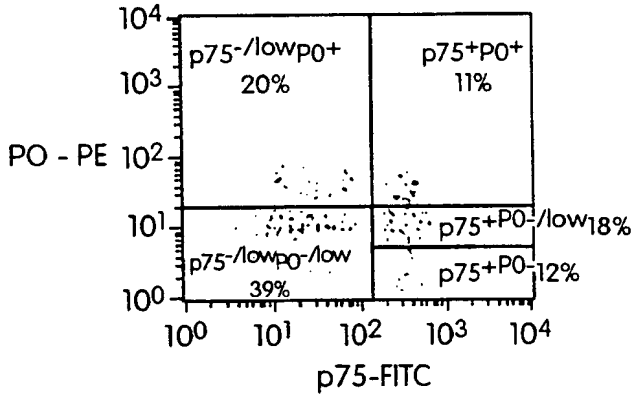
UNSTAINED
TRYPSIN + COLLAGENASE

Fig. 1A



STAINED
HYALURONIDASE + COLLAGENASE

Fig. 1B



STAINED
TRYPSIN + COLLAGENASAE

Fig. 1C

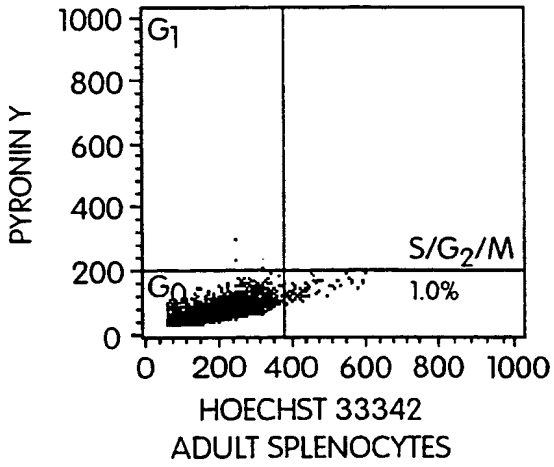


Fig. 2A

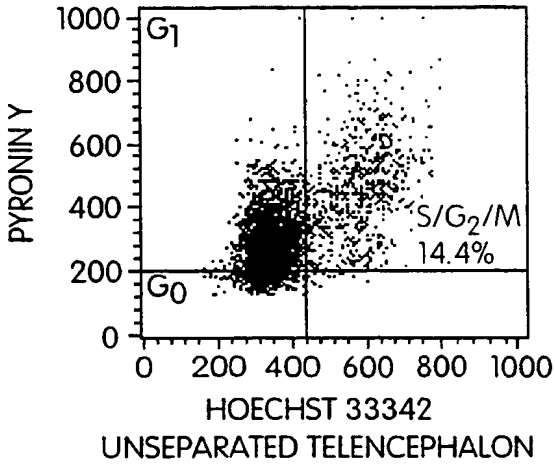


Fig. 2B

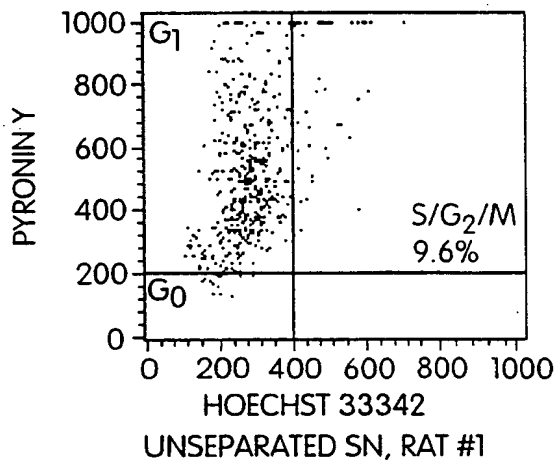


Fig. 2C

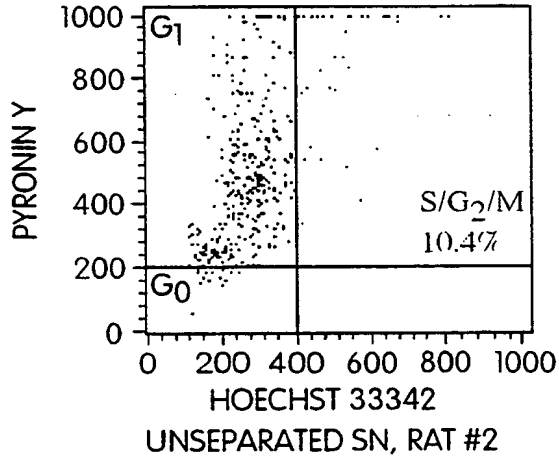


Fig. 2D

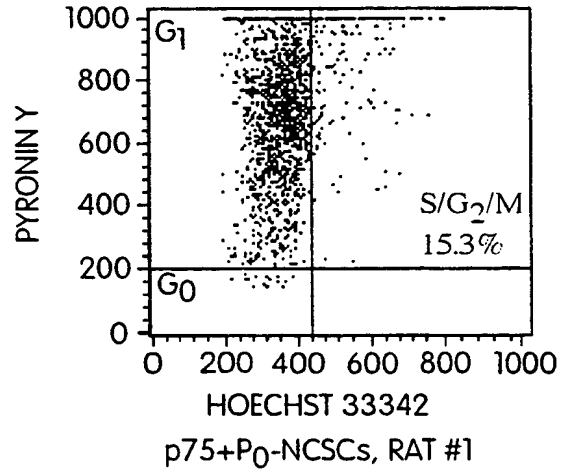


Fig. 2E

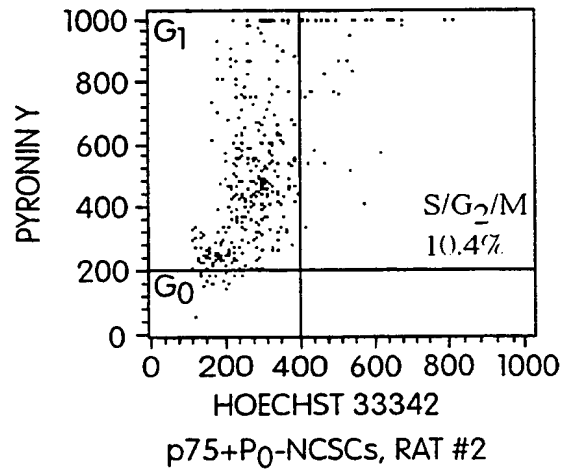


Fig. 2F

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